MycoKeys

MycoKeys 121: 237–251 (2025)
DOI: 10.3897/mycokeys.121.158721

Research Article

Morphological and phylogenetic analyses reveal two new species of *Neohelicomyces* (Tubeufiales, Tubeufiaceae) from China

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Abstract

Neohelicomyces is a genus of helicosporous hyphomycetes with the potential to produce bioactive secondary metabolites. During a survey of helicosporous fungi in Guizhou and Hainan provinces, southern China, four isolates were obtained from both freshwater and terrestrial habitats. Based on combined analyses of multigene phylogenetic data (ITS, LSU, *tef*1-α, and *rpb*2) and morphological characteristics, two novel species, *Neohelicomyces aquisubtropicus* and *N. wuzhishanensis*, are proposed. Detailed descriptions, illustrations, and phylogenetic analyses of the new taxa are presented. Additionally, a checklist of currently accepted *Neohelicomyces* species supported by molecular data is provided.

Key words: 2 new species, asexual morph, Dothideomycetes, hyphomycetes, taxonomy

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This article is part of:
Exploring the Hidden Fungal Diversity: Biodiversity, Taxonomy, and Phylogeny of Saprobic Fungi
Edited by Samantha C. Karunarathna, Danushka Sandaruwan Tennakoon, Ajay Kumar Gautam

Academic editor:
Samantha C. Karunarathna
Received: 12 May 2025
Accepted: 2 July 2025
Published: 29 August 2025

Citation: Ma X-Y, Song D-D, Ma J (2025) Morphological and phylogenetic analyses reveal two new species of *Neohelicomyces* (Tubeufiales, Tubeufiaceae) from China. MycoKeys 121: 237–251. https://doi.org/10.3897/mycokeys.121.158721

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Introduction

Luo et al. (2017) introduced the genus *Neohelicomyces* and designated *N. aquaticus* as the type species based on phylogenetic analysis of a combined dataset (*ITS*, *LSU*, and *tef*1-α) and morphological features. The asexual morph of *Neohelicomyces* is characterized by gregarious colonies that are white, grayish-brown, yellowish-green, or pinkish; macronematous, mononematous, erect, septate, pale brown, branched and/or unbranched conidiophores; mono- to polyblastic, denticulate, integrated, terminal or intercalary conidiogenous cells; and acropleurogenous or pleurogenous, aseptate or septate, guttulate, hyaline, helicoid conidia (Lu et al. 2018, 2022; Tibpromma et al. 2018; Crous et al. 2019a,b; Dong et al. 2020; Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024a, b; Peng et al. 2025).

Currently, *Neohelicomyces* comprises 28 species (Yang et al. 2023; Ma et al. 2024b; Peng et al. 2025; Sun et al. 2025). Among them, 12 species are found in freshwater habitats, 12 in terrestrial habitats, and four in both freshwater and terrestrial habitats (Table 1). *Neohelicomyces* species are distributed in China, the Czech Republic, Germany, Italy, Japan, the Netherlands, Thailand, and the USA (Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024b; Peng et al. 2025). They occur as saprobes on bamboo culms, *Deschampsia cespitosa*,

Table 1. A checklist of accepted *Neohelicomyces* species with molecular data.

No.	Species	Distribution	Habitat	Reference
1	*Neohelicomyces acropleurogenus*	China	Terrestrial	Ma et al. (2024b)
2	*Neohelicomyces aquaticus*	China	Freshwater	Luo et al. (2017)
3	***Neohelicomyces aquisubtropicus***	**China**	**Terrestrial**	**This study**
4	*Neohelicomyces aseptatus*	China	Terrestrial	Ma et al. (2024b)
5	*Neohelicomyces dehongensis*	China	Freshwater	Dong et al. (2020)
6	*Neohelicomyces denticulatus*	China	Freshwater	Yang et al. (2023)
7	*Neohelicomyces deschampsiae*	Germany	Terrestrial	Crous et al. (2019a)
8	*Neohelicomyces edgeworthiae*	China	Terrestrial	Ma et al. (2024b)
9	*Neohelicomyces guizhouensis*	China	Freshwater	Ma et al. (2024a)
10	*Neohelicomyces guttulatus*	China	Freshwater/ Terrestrial	Ma et al. (2024b)
11	*Neohelicomyces grandisporus*	China	Freshwater	Luo et al. (2017)
12	*Neohelicomyces hainanensis*	China	Terrestrial	Lu et al. (2022)
13	*Neohelicomyces helicosporus*	China	Terrestrial	Ma et al. (2024a)
14	*Neohelicomyces hyalosporus*	China	Freshwater	Lu et al. (2018)
15	*Neohelicomyces hydei*	China	Freshwater	Ma et al. (2024a)
16	*Neohelicomyces lignicola*	China	Freshwater	Ma et al. (2024b)
17	*Neohelicomyces longisetosus*	China	Freshwater	Hsieh et al. (2021)
18	*Neohelicomyces macrosporus*	China	Freshwater	Ma et al. (2024b)
19	*Neohelicomyces maolanensis*	China	Terrestrial	Peng et al. (2025)
20	*Neohelicomyces melaleucae*	China, USA	Freshwater/ Terrestrial	Crous et al. (2019b)
21	*Neohelicomyces pallidus*	China, Czech Republic, Italy, Japan, Netherlands, USA	Freshwater/ Terrestrial	Linder (1929); Goos (1989); Tsui et al. (2001); Zhao et al. (2007); Lu et al. (2018); Ma et al. (2024b)
22	*Neohelicomyces pandanicola*	China	Terrestrial	Tibpromma et al. (2018)
23	*Neohelicomyces qixingyansis*	China	Terrestrial	Ma et al. (2024b)
	Neohelicomyces sexualis	China	Terrestrial	Sun et al. (2025)
24	*Neohelicomyces submersus*	China	Freshwater	Luo et al. (2017)
25	*Neohelicomyces subtropicus*	China	Terrestrial	Peng et al. (2025)
26	*Neohelicomyces thailandicus*	China, Thailand	Freshwater/ Terrestrial	Dong et al. (2020); Ma et al. (2024b)
27	***Neohelicomyces wuzhishanensis***	**China**	**Freshwater**	**This study**
28	*Neohelicomyces xiayadongus*	China	Terrestrial	Ma et al. (2024b)
29	*Neohelicomyces yunnanensis*	China	Freshwater	Ma et al. (2024b)

Note: The newly isolated species in this study are highlighted in bold.

Fraxinus excelsior, *Melaleuca styphelioides*, *Miscanthus floridulus*, *Pandanus* sp., *Quercus robur*, and decaying wood in both freshwater and terrestrial habitats (Linder 1929; Goos 1985, 1986, 1989; Tsui et al. 2006; Zhao et al. 2007; Ruibal et al. 2009; Luo et al. 2017; Lu et al. 2018, 2022; Tibpromma et al. 2018; Crous et al. 2019a, b; Dong et al. 2020; Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024b; Peng et al. 2025; Sun et al. 2025).

Neohelicomyces has the potential to produce secondary metabolites (Zheng et al. 2023). For example, two alkaloid compounds isolated from *Neohelicomyces hyalosporus* exhibited moderate cytotoxic effects on human cancer cells (Zheng et al. 2023).

In this study, four helicosporous hyphomycete isolates, representing two distinct taxa, were obtained from both freshwater and terrestrial habitats in

Guizhou and Hainan provinces, China. Based on morphological characteristics, illustrations, and multigene phylogenetic analyses, two novel species are introduced: *Neohelicomyces aquisubtropicus* and *N. wuzhishanensis*.

Materials and methods

Sample collection, examination, and isolation

Decaying wood samples were collected from freshwater and terrestrial habitats in Guizhou and Hainan provinces, China, from August 2021 to April 2022. After the collection information was recorded (Rathnayaka et al. 2024), the fungal specimens were taken to the mycology laboratory at the Guizhou Institute of Technology for examination. Fresh specimens from freshwater habitats were cultured at room temperature, with moisture maintained for 1–2 weeks. Morphological characteristics were observed using a stereomicroscope (SMZ-168, Nikon, Japan) and photographed using an ECLIPSE Ni compound microscope (Nikon, Tokyo, Japan) equipped with a Canon 90D digital camera.

Single-spore isolations were conducted following the procedure outlined in Senanayake et al. (2020). Subsequently, the germinating spores were aseptically transferred to fresh potato dextrose agar (PDA). Dried specimens were deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences (Herb. HKAS), Kunming, China, and the Herbarium of Guizhou Academy of Agriculture Sciences (Herb. GZAAS), Guiyang, China. Pure cultures were deposited at the Guizhou Culture Collection (**GZCC**), Guiyang, China. The MycoBank numbers were obtained as described at https://www.mycobank.org/.

DNA extraction, PCR amplification, and sequencing

Fresh fungal mycelia grown on PDA media for 39–45 days were scraped with a sterilized toothpick and transferred to a 1.5 ml microcentrifuge tube for genomic DNA extraction. Genomic DNA was extracted using the Biospin Fungus Genomic DNA Extraction Kit (BioFlux, China), following the manufacturer's protocol. Primer pairs ITS5/ITS4 (White et al. 1990), LR0R/LR5 (Vilgalys and Hester 1990), EF1-983F/EF1-2218R (Rehner and Buckley 2005), and fRPB2-5F/fRPB2-7cR (Liu et al. 1999) were used to amplify ITS, LSU, *tef*1-α, and *rpb*2 sequence fragments, respectively. The PCR amplification reactions were carried out in a 25 μL reaction volume, including 1 μL of DNA, 1 μL each of the forward and reverse primers, and 22 μL of 1.1× T3 Super PCR Mix (Qingke Biotech, Chongqing, China). The polymerase chain reaction (PCR) followed the protocol reported by Ma et al. (2024a). The PCR products were detected by 1% agarose gel electrophoresis, and sequencing was performed at Beijing Tsingke Biotechnology Co., Ltd.

Phylogenetic analyses

The forward and reverse sequence data of the new taxa were checked and assembled using BioEdit v. 7.0.5.3 (Hall 1999) and SeqMan v. 7.0.0 (DNASTAR, Madison, WI, USA; Swindell and Plasterer 1997), respectively. The sequences used in this study were downloaded from GenBank (Table 2; https://www.ncbi.nlm.nih.gov/). The single-gene datasets (ITS, LSU, *tef*1-α, and *rpb*2) were

Table 2. Taxa used in this study, along with their corresponding GenBank accession numbers.

Taxon	Strain	GenBank Accessions			
		ITS	LSU	*tef*1-α	*rpb*2
Helicotubeufia hydei	MFLUCC 17-1980^{T}	MH290021	MH290026	MH290031	MH290036
Helicotubeufia jonesii	MFLUCC 17-0043^{T}	MH290020	MH290025	MH290030	MH290035
Muripulchra aquatica	MFLUCC 15-0249^{T}	KY320532	KY320549	-	-
Neohelicomyces acropleurogenus	CGMCC 3.25549^{T}	PP626594	PP639450	PP596351	PP596478
Neohelicomyces aquisubtropicus	**GZCC 23-0080^{T}**	**PQ098499**	**PQ098537**	**PV768327**	**PV768336**
Neohelicomyces aquisubtropicus	**GZCC 24-0163**	**PV730410**	**PV730414**	**PV768328**	**PV768337**
Neohelicomyces aquaticus	MFLUCC 16-0993^{T}	KY320528	KY320545	KY320561	MH551066
Neohelicomyces aseptatus	CGMCC 3.25564^{T}	PP626595	PP639451	PP596352	PP596479
Neohelicomyces dehongensis	MFLUCC 18-1029^{T}	NR_171880	MN913709	MT954393	-
Neohelicomyces denticulatus	GZCC 19-0444^{T}	OP377832	MW133855	-	-
Neohelicomyces deschampsiae	CPC 33686^{T}	MK442602	MK442538	-	-
Neohelicomyces edgeworthiae	CGMCC 3.25565^{T}	PP626597	PP639453	PP596354	PP596481
Neohelicomyces grandisporus	KUMCC 15-0470^{T}	KX454173	KX454174	-	MH551067
Neohelicomyces guizhouensis	GZCC 23-0725^{T}	PP512969	PP512973	PP526727	PP526733
Neohelicomyces guttulatus	CGMCC 3.25550^{T}	PP626598	PP639454	PP596355	-
Neohelicomyces hainanensis	GZCC 22-2009^{T}	OP508734	OP508774	OP698085	OP698074
Neohelicomyces helicosporus	GZCC 23-0633^{T}	PP512971	PP512975	PP526729	PP526735
Neohelicomyces hyalosporus	GZCC 16-0086^{T}	MH558745	MH558870	MH550936	MH551064
Neohelicomyces hydei	GZCC 23-0727^{T}	-	PP512977	PP526731	PP526737
Neohelicomyces lignicola	CGMCC 3.25551^{T}	PP626600	PP639456	PP596357	PP596483
Neohelicomyces longisetosus	NCYU-106H1-1-1^{T}	MT939303	-	-	-
Neohelicomyces macrosporus	CGMCC 3.25552^{T}	PP626601	PP639457	PP596358	PP596484
Neohelicomyces maolanensis	GZCC 23-0079^{T}	-	PQ098529	PQ490683	PQ490677
Neohelicomyces melaleucae	CPC 38042^{T}	MN562154	MN567661	MN556835	-
Neohelicomyces pallidus	CBS 271.52	AY916461	AY856887	-	-
Neohelicomyces pallidus	CBS 962.69	AY916460	AY856886	-	-
Neohelicomyces pandanicola	KUMCC 16-0143^{T}	MH275073	MH260307	MH412779	-
Neohelicomyces qixingyaensis	CGMCC 3.25569^{T}	PP626602	PP639458	PP596359	PP596485
Neohelicomyces submersus	MFLUCC 16-1106^{T}	KY320530	KY320547	-	MH551068
Neohelicomyces subtropicus	GZCC 23-0076^{T}	PQ098492	PQ098530	PQ490685	PQ490679
Neohelicomyces thailandicus	MFLUCC 11-0005^{T}	NR_171882	MN913696	-	-
Neohelicomyces wuzhishanensis	**GZCC 23-0410^{T}**	**PQ098494**	**PQ098532**	**PV768325**	**PV768334**
Neohelicomyces wuzhishanensis	**GZCC 24-0164**	**PV730409**	**PV730413**	**PV768326**	**PV768335**
Neohelicomyces xiayadongensis	CGMCC 3.25568^{T}	PP626604	PP639460	PP596361	PP596487
Neohelicomyces yunnanensis	GZCC 23-0735^{T}	PP664109	PP664113	-	-
Tubeufia guttulata	GZCC 23-0404^{T}	OR030841	OR030834	OR046678	OR046684
Tubeufia hainanensis	GZCC 22-2015^{T}	OR030842	OR030835	OR046679	OR046685
Tubeufia javanica	MFLUCC 12-0545^{T}	KJ880034	KJ880036	KJ880037	-
Tubeufia krabiensis	MFLUCC 16-0228^{T}	MH558792	MH558917	MH550985	MH551118
Tubeufia latispora	MFLUCC 16-0027^{T}	KY092417	KY092412	KY117033	MH551119
Tubeufia laxispora	MFLUCC 16-0232^{T}	KY092413	KY092408	KY117029	MF535287
Tubeufia mackenziei	MFLUCC 16-0222^{T}	KY092415	KY092410	KY117031	MF535288
Tubeufia muriformis	GZCC 22-2039^{T}	OR030843	OR030836	OR046680	OR046686
Tubeufia nigroseptum	CGMCC 3.20430^{T}	MZ092716	MZ853187	OM022002	OM022001
Tubeufia pandanicola	MFLUCC 16-0321^{T}	MH275091	MH260325	-	-
Tubeufiaceae sp.	ATCC 42524	AY916458	AY856911	-	-

Note: "^{T}" indicates ex-type strains. Newly generated sequences are in bold. "-" indicates the unavailable data in GenBank.

aligned using MAFFT v. 7.473 (https://mafft.cbrc.jp/alignment/server/, Katoh et al. 2019) and trimmed using trimAl v.1.2rev59 software (Capella-Gutiérrez et al. 2009). The aligned datasets (LSU–ITS–*tef*1-α–*rpb*2) were concatenated using SequenceMatrix-Windows v. 1.7.8 software (Vaidya et al. 2011). The maximum likelihood (ML) tree was constructed using the IQ-TREE webserver (http://iqtree.cibiv.univie.ac.at/, Nguyen et al. 2015).

Bayesian analyses were carried out using MrBayes v. 3.2.7a, and the best nucleotide substitution model for each data partition was selected using MrModeltest v. 2.3 under the Akaike Information Criterion (AIC) (Nylander et al. 2008). The aligned FASTA file was converted to a NEXUS format for Bayesian analysis using AliView v. 1.27 (Larsson 2014).

Phylogenetic trees were visualized using FigTree v. 1.4.4 and subsequently edited in Adobe Illustrator CC 2019 (v. 23.1.0; Adobe Systems, USA). Photo plates and scale bars were prepared using Adobe Photoshop CC 2019 (Adobe Systems, USA) and the Tarosoft Image Framework program, respectively.

Phylogenetic analysis results

The phylogenetic placements of the four new strains were determined by multilocus phylogenetic analysis. The concatenated sequence matrix comprised 3,410 characters (ITS: 1–573, LSU: 574–1,430, *tef*1-α: 1,431–2,341, and *rpb*2: 2,342–3,410) across 46 taxa. Both ML and BI analyses produced congruent topologies. Fig. 1 presents the best-scoring ML tree, which had a final log-likelihood value of –19,563.192. Species delimitation and the introduction of new taxa were conducted following the taxonomic framework proposed by Chethana et al. (2021).

Based on the multigene phylogenetic tree (Fig. 1), our collections represent two distinct species of *Neohelicomyces* within the family Tubeufiaceae. Isolates GZCC 23-0080 and GZCC 24-0163 formed a sister clade to the clade comprising *Neohelicomyces denticulatus* (GZCC 19-0444), *N. edgeworthiae* (CGMCC 3.25565), and *N. pandanicola* (KUMCC 16-0143). Isolates GZCC 23-0410 and GZCC 24-0164 formed a clade that is sister to *Neohelicomyces guizhouensis* (GZCC 23-0725) with 92% ML bootstrap support.

Taxonomy

Neohelicomyces aquisubtropicus **X.Y. Ma, Y.Z. Lu & J. Ma, sp. nov.**

MycoBank No: 904050

Fig. 2

Etymology. "aqui-" refers to the aquatic habitat of this fungus, and "-subtropicus" means the climate type where the fungus was collected.

Holotype. HKAS 128947

Description. ***Saprobic*** on decaying wood in a terrestrial habitat. ***Sexual morph*** Undetermined. ***Asexual morph*** Hyphomycetous, helicosporous. ***Colonies*** on natural substrate superficial, white, effuse, gregarious, with massive glistening conidia. ***Mycelium*** partly superficial, composed of hyaline to pale brown, branched, septate, guttulate, smooth hyphae. ***Conidiophores*** 144–193.5 × 3.5–6.5 µm ($\overline{x}$ = 167.5 × 5 µm, n = 25), macronematous, mononematous, erect, cylindrical, straight or slightly flexuous, typically curved at the apex, unbranched,

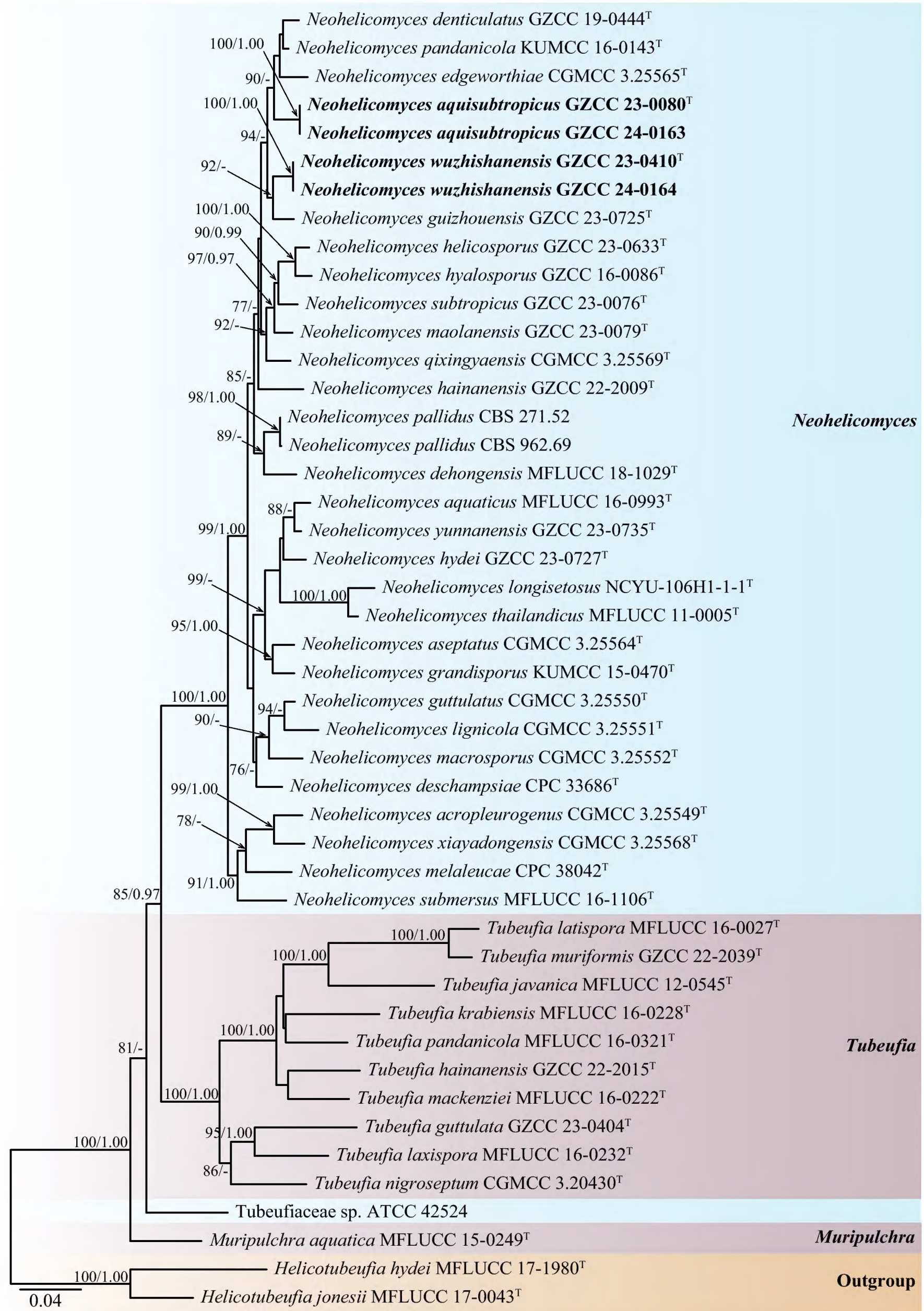

Figure 1. Phylogenetic tree generated from ML analysis based on the combined ITS, LSU, *tef*1-α, and *rpb*2 sequence data. Bootstrap support values for ML (≥ 75%) and BI (≥ 0.95) are indicated near their respective nodes. The tree is rooted with *Helicotubeufia hydei* (MFLUCC 17-1980) and *H. jonesii* (MFLUCC 17-0043). Ex-type strains are denoted with "T," and newly obtained strains are in bold black fonts.

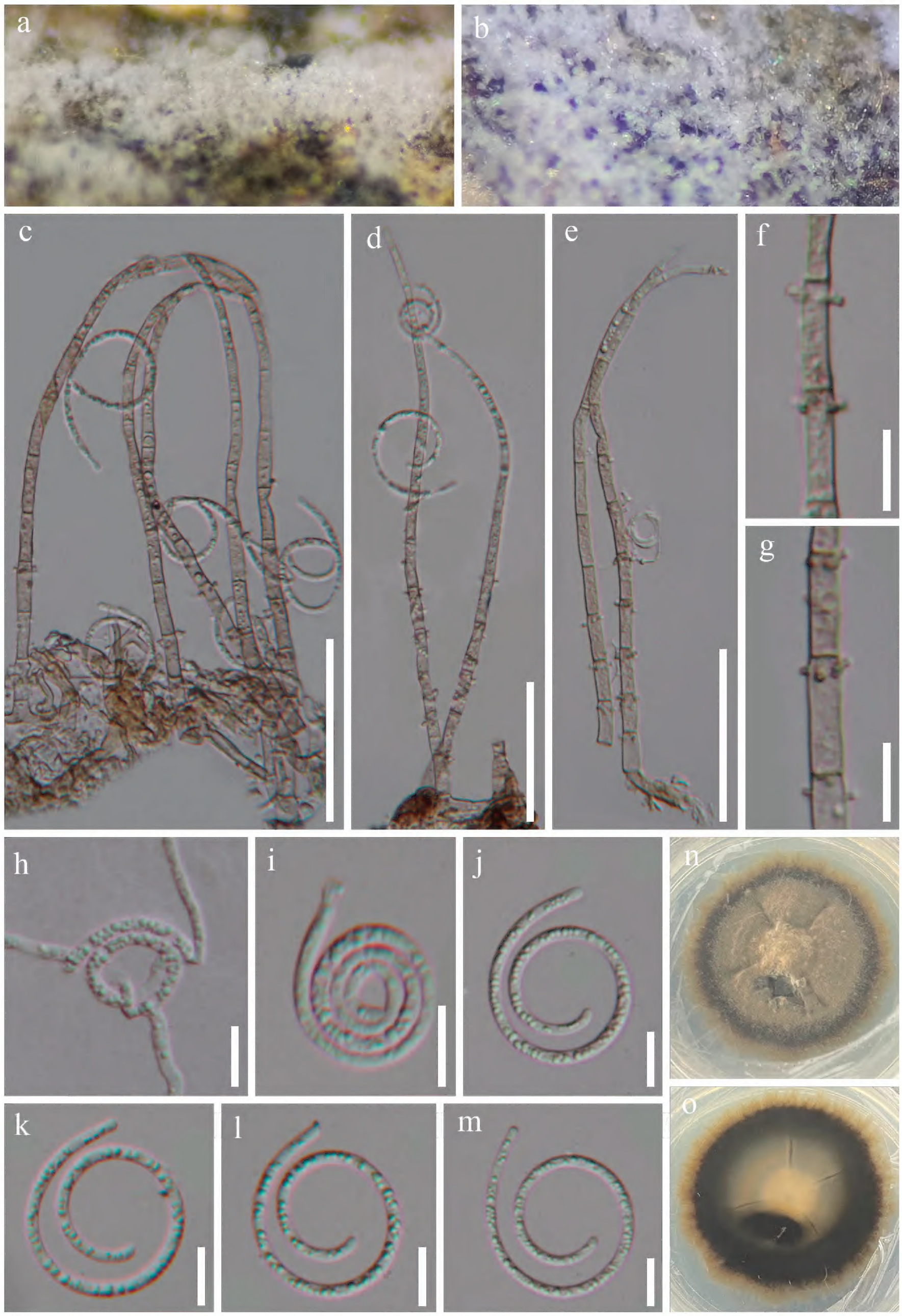

Figure 2. *Neohelicomyces aquisubtropicus* (HKAS 128947, holotype). **a, b.** Colonies on the host surface; **c–e.** Conidiophores, conidiogenous cells, and conidia; **f, g.** Conidiogenous cells; **h.** Germinated conidium; **i–m.** Conidia; **n, o.** Colonies on PDA from above and below after 45 days of incubation at room temperature. Scale bars: 20 μm (**c–e**); 10 μm (**f–m**).

septate, subhyaline to pale brown, thick-walled. ***Conidiogenous cells*** 11.5–15 × 3.5–5 µm ($\overline{x}$ = 13.5 × 4 µm, n = 30), holoblastic, monoblastic, or polyblastic, integrated, intercalary, cylindrical, with denticles, subhyaline to pale brown, smooth-walled. ***Conidia*** solitary, pleurogenous, helicoid, tapering towards the rounded ends, developing on tooth-like protrusions, 14.5–17 µm diam., and conidial filament 2–4 µm wide ($\overline{x}$ = 15.5 × 3 µm, n = 25), 82.5–126.5 µm long ($\overline{x}$ = 105.5 µm, n = 30), tightly coiled up to $3^1/_2$ times, becoming loosely coiled in water, aseptate, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia germinate on PDA within 10 hours, producing germ tubes from the conidial body. Colonies on PDA are circular with a raised surface and entire margin, reaching 5 cm in diameter after 45 days at room temperature (approximately 25 °C), and are pale brown to dark brown on both the surface and reverse sides.

Material examined. China • Guizhou Province, Qiannan Buyi and Miao Autonomous Prefecture, Libo County, on decaying wood in a terrestrial habitat, 10 April 2022, Jian Ma, MN6 (HKAS 128947, holotype), ex-type living cultures GZCC 23-0080; • *Ibid*., MN6.1 (GZAAS 24-0077, paratype), living culture GZCC 24-0163.

Notes. Based on phylogenetic analyses, our isolates (GZCC 23-0080 and GZCC 24-0163) clustered with *Neohelicomyces denticulatus* (GZCC 19-0444), *N. edgeworthiae* (CGMCC 3.25565), and *N. pandanicola* (KUMCC 16-0143) (Fig. 1). *Neohelicomyces aquisubtropicus* (HKAS 128947) differs from *N. edgeworthiae* (HKAS 128877) in having smaller conidia (14.5–17 µm diam. and 82.5–126.5 µm long *vs.* 21.5–34 µm diam. and 121–177 µm long) (Ma et al. 2024b). Additionally, *N. denticulatus* (GZAAS 20-0339) and *N. pandanicola* (HKAS 96202) can be distinguished from *N. aquisubtropicus* (HKAS 128947) by their wider conidial diameters (16–22 µm and 28–44 µm *vs*. 14.5–17 µm) (Tibpromma et al. 2018; Yang et al. 2023). Moreover, base pair comparisons between *N. aquisubtropicus* (GZCC 23-0080) and related species reveal the following differences. Compared to *N. denticulatus* (GZCC 19-0444), there are 23/487 bp differences in ITS (4.7%, with 13 gaps). Compared to *N. edgeworthiae* (CGMCC 3.25565), there are 26/521 bp differences in ITS (5.0%, with 14 gaps), 21/929 bp differences in *tef*1-α (2.3%, with 7 gaps), and 27/811 bp differences in *rpb*2 (3.3%, with 10 gaps). In comparison with *N. pandanicola* (KUMCC 16-0143), there are 27/509 bp differences in ITS (5.3%, with 13 gaps) and 15/827 bp differences in *tef*1-α (1.8%, with 7 gaps). Therefore, we introduce *N. aquisubtropicus* as a new species based on morphology and multigene phylogenetic analysis.

Neohelicomyces wuzhishanensis **X.Y. Ma, Y.Z. Lu & J. Ma, sp. nov.**

MycoBank No: 904051

Fig. 3

Etymology. "*wuzhishanensis*" refers to the type location "Wuzhishan National Nature Reserve."

Holotype. HKAS 128942

Description. ***Saprobic*** on submerged decaying wood in a freshwater habitat. ***Sexual morph*** Undetermined. ***Asexual morph*** Hyphomycetous, helicosporous. ***Colonies*** on natural substrate superficial, effuse, gregarious, white. ***Mycelium*** partly immersed, composed of hyaline to pale brown, branched, septate, guttulate,

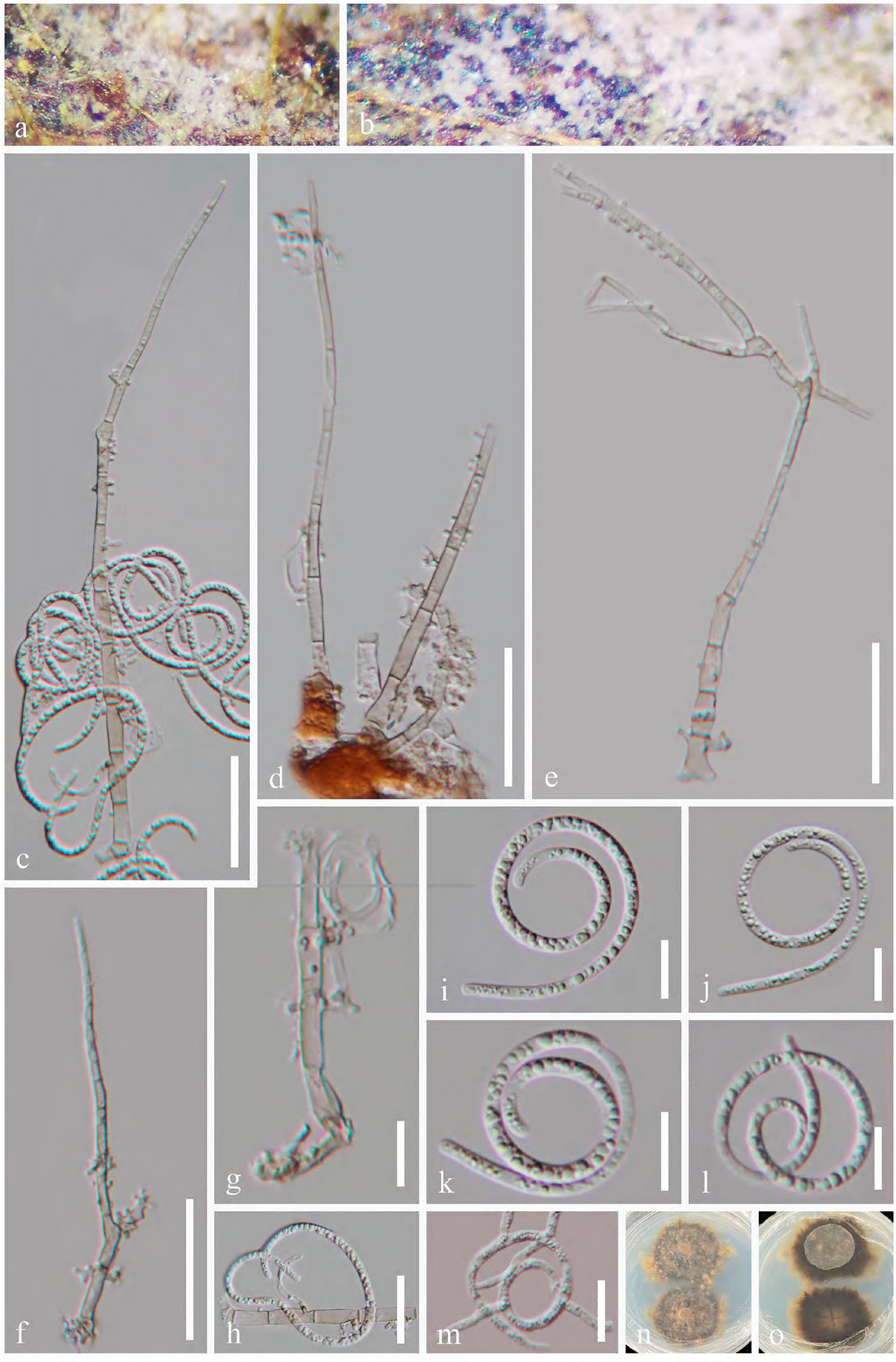

Figure 3. *Neohelicomyces wuzhishanensis* (HKAS 128942, holotype). **a, b.** Colonies on the host surface; **c–h.** Conidiophores, conidiogenous cells, and conidia; **i–l.** Conidia; **m.** A germinated conidium; **n, o.** Colonies on PDA from above and below after 39 days of incubation at room temperature. Scale bars: 30 μm (**c–f**); 20 μm (**h, m**); 10 μm (**g, i–l**).

smooth hyphae. ***Conidiophores*** 92–190 × 3.5–5 µm ($\overline{x}$ = 140 × 4.5 µm, n = 25), macronematous, mononematous, erect, cylindrical, widest at the base, tapering towards narrow apex, straight or slightly flexuous, occasionally branched, septate, subhyaline to pale brown, thick-walled. ***Conidiogenous cells*** 9.5–16.5 × 2.5–5 µm ($\overline{x}$ = 14 × 4 µm, n = 20), holoblastic, monoblastic, or polyblastic, integrated, intercalary or terminal, cylindrical, with tiny tooth-like or bladder-like protrusions, subhyaline to pale brown, smooth-walled. ***Conidia*** solitary, acropleurogenous, helicoid, tapering towards the rounded ends, developing on tooth-like protrusions, 23–26 µm diam., and conidial filament 2.3–3.5 µm wide ($\overline{x}$ = 24.5 × 2.8 µm, n = 20), 118–143.5 µm long ($\overline{x}$ = 129 µm, n = 20), tightly coiled 1.5–2 times, becoming loosely coiled in water, aseptate, guttulate, hyaline, smooth-walled.

Culture characteristics. Conidia germinate on PDA within 14 hours, producing germ tubes from the conidial body. Colonies on PDA are irregular with a raised surface and undulate margin, reaching 3 cm in diameter after 39 days at room temperature (approximately 25 °C), and are brown to dark brown on both the surface and reverse sides.

Material examined. China • Hainan Province, Wuzhishan City, Shuimanhe tropical rainforest scenic area in Wuzhishan, 18°92'N, 109°63'E, on rotting wood in a freshwater habitat, 15 August 2021, Jian Ma, WZS8.2 (HKAS 128942, holotype), ex-type living cultures GZCC 23-0410; • *Ibid*., WZS8.5 (GZAAS 24-0078, paratype), living culture GZCC 24-0164.

Notes. In our phylogenetic tree (Fig. 1), our isolates (GZCC 23-0410 and GZCC 24-0164) formed a sister clade to *N. guizhouensis* (GZCC 23-0725) with 92% ML bootstrap support. *Neohelicomyces wuzhishanensis* (HKAS 128942) can be distinguished from *N. guizhouensis* (KAS 134924) by its wider conidial diameters (23–26 µm *vs*. 18–21.5 µm) (Ma et al. 2024a). Moreover, base pair comparison of *N. wuzhishanensis* (GZCC 23-0410) and *N. guizhouensis* (GZCC 23-0725) shows 31/539 bp differences in ITS (5.8%, gaps 13 bp), 4/530 bp differences in LSU (0.8%, gaps 3 bp), 13/877 bp differences in *tef*1-α (1.5%), and 23/939 bp differences in *rpb*2 (2.4%). Therefore, based on the multigene phylogenetic analysis and morphological differences, we introduce *N. wuzhishanensis* as a novel species.

Discussion

Neohelicomyces currently comprises 30 species, including the two newly described species, *N. aquisubtropicus* and *N. wuzhishanensis* (Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024a, b; Peng et al. 2025; Sun et al. 2025).

According to previously published studies, six genera—*Helicodendron*, *Helicoma*, *Helicosporium*, *Neohelicomyces*, *Neohelicosporium*, and *Tubeufia*—represent the most species-rich genera among helicosporous hyphomycetes (Lu and Kang 2020, 2022; Ma et al. 2024a, b; Lu et al. 2025; Peng et al. 2025). The asexual morph of most *Neohelicomyces* species closely resembles that of *Helicomyces*, *Pseudotubeufia*, and *Tubeufia* in having mononematous, septate, pale brown conidiophores; mono- to polyblastic conidiogenous cells; and acropleurogenous or pleurogenous, aseptate or septate, hyaline, helicoid conidia (Yang et al. 2023; Ma et al. 2023, 2024a, b; Peng et al. 2025; Sun et al. 2025). For these morphologically similar helicosporous genera, accurate identification requires a combination of multigene phylogenetic analyses and detailed morphological examination (Linder 1929; Goos 1985, 1986, 1989; Tsui et al. 2006; Zhao et al.

2007; Ruibal et al. 2009; Hyde et al. 2016; Luo et al. 2017; Lu et al. 2018, 2022; Tibpromma et al. 2018; Crous et al. 2019a, b; Dong et al. 2020; Boonmee et al. 2011, 2014, 2021; Hsieh et al. 2021; Yang et al. 2023; Ma et al. 2024a, b; Peng et al. 2025). It is important to note that comparisons of conidial diameter and number of coils among helicosporous species should be based on tightly coiled conidia to ensure consistency and accuracy (Lu et al. 2022, 2023a, b; Xiao et al. 2023; Yang et al. 2023; Ma et al. 2024a, b; Peng et al. 2025).

Some *Neohelicomyces* species exhibit morphological variations across different habitats and geographical regions (Linder 1929; Goos 1989; Tsui et al. 2001; Zhao et al. 2007; Lu et al. 2018; Yang et al. 2023; Ma et al. 2024b). For example, two collections—HMAS 98776 from a terrestrial habitat in Hebei Province and GZAAS 20-0339 from a freshwater habitat in Guizhou Province, China—both identified as *Neohelicomyces pallidus*, show distinct morphological characters (Zhao et al. 2007; Yang et al. 2023). HMAS 98776 differs from GZAAS 20-0339 by possessing smaller conidia (10–16 µm vs. 16–22 µm) and exclusively intercalary conidiogenous cells, whereas GZAAS 20-0339 exhibits both terminal and intercalary conidiogenous cells (Zhao et al. 2007; Yang et al. 2023). These morphological differences are speculated to result from environmental variation across habitats and geographic locations. Therefore, species identification within this genus primarily relies on molecular data, which serve as the principal criterion in taxonomic decision-making. This study enriches our understanding of fungal diversity in subtropical and tropical ecosystems and provides cultures of fungal strains for subsequent research on the secondary metabolites of *Neohelicomyces*.

Acknowledgments

We would like to thank Shaun Pennycook (Manaaki Whenua Landcare Research, New Zealand) for his valuable suggestions on the Latin names of the new species.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This work was funded by the Guizhou Institute of Technology High-Level Talent Scientific Research Start-Up Fund (grant number 2023GCC066), the Guizhou Provincial Higher Education Undergraduate Teaching Content and Curriculum System Reform Project (grant number GZJG2024209), the Zunyi Technology and Big Data Bureau–Moutai Institute Joint Science and Technology Research and Development Project (grant number ZunShiJiaoHe HZ zi[2023]110), and the Science and Technology Foundation of Guizhou Province (Qian Ke He Pingtai ZSYS[2025]029).

Author contributions

Morphological data, photo-plates and phylogenetic analyzes were completed by Jian Ma and Xiao-Yan Ma. The original draft was written by Jian Ma, and Xiao-Yan Ma and Dan-Dan Song revised the paper.

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Data availability

All of the data that support the findings of this study are available in the main text.

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